Testing the Effects of Oxidative Stress on *Escherichia coli*

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Abstract

There has been previous research about the location of DNA transcription promoters and their roles in cells, such as the model organism and bacterial cell *Escherichia coli* K-12, when undergone different types of shock, stress, or nutrient limitation. *E.coli* K-12 is a model organism for scientists, used to determine the reactions of protein synthesis when treated with stressors such as heat shock, nutrient starvation and DNA-damaging agents. In this laboratory investigation, we will investigate how a normal strand of *E. coli* reacts to oxidative stress with hydrogen peroxide, and compare its reaction to the oxidative stress reaction of a mutant strand of *E. coli*. After analyzing the two reactions, the online software, OK Mapper will be used to locate the promoters of the control group, that were not able to respond to the oxidative stress response with hydrogen peroxide, and in response, locate the promoters that are involved in oxidative stress response with hydrogen peroxide.

Keywords: *Escherichia coli*, DNA, Oxidative Stress

Introduction

The growth conditions that lead to an imbalance in Reactive Oxygen Species (ROS), such as superoxide anion (O_2 -), hydroxyl radical (HO), or hydrogen peroxide (H_2O_2), result in oxidative stress (Figure 1). As seen in Figure 1, ROS molecules can cause irreparable damage to the cell membrane, proteins and DNA leading to cell death if they are not neutralized (Imlay 2013). The oxidant-generating defense mechanisms have been identified in numerous plants and animals, but regulation of the response to H_2O_2 has been studied most comprehensively in Escherichia coli, and is regulated by the activator, OxyR (Pomposiello *et al.* 2001). Research done by Greenberg and Demple demonstrated that a significant overlap exists between the protein expression profiles of E. coli treated with menadione (vitamin K3) and H_2O_2, it also suggested that the *E. coli* oxidative stress response is regulated by multiple pathways (Greenberg *et al.* 1989). However, the signaling pathways that are upregulated by H_2O_2 are poorly understood. Our aim in this investigation is to utilize Differential RNA Sequencing (dRNA-seq) to capture a glimpse of the *E. coli* global response to oxidative stress elicited by H_2O_2. Data from this work will highlight H_2O_2- specific promoter activation, and signal networks that are critical to neutralizing ROS, and those networks also critical to restoring intracellular homeostasis.

Methods

Wild-type (WT) *E. coli* (BW38028) was grown in a MOPS [3-(N-morpholino) propanesulfonic acid] minimal medium containing 0.05% glucose at 37° ± 0.3 ℃ with shaking (250 rpm) for approximately 16 hours. The optical density at 600nm (OD600) of each culture was measured using a Biophotometer D30 (Eppendorf), and the cultures were diluted into MOPS containing 0.2% glucose allowed to grow for 10 generations. Immediately prior to reaching the mid-log phase of bacterial growth (OD600 ≅ 0.5), H_2O_2 was added to each 50 mL culture to the desired final concentration (50 µg/ml or 200 µg/ml). After 30 minutes, 1.0 mL of each culture was removed and dispensed into an RNAlater stabilization solution to rapidly deactivate any RNAses and preserve RNA integrity for downstream analyses. *E. coli* cells were pelleted using a Microfuge®18 Microcentrifuge (Beckman Coulter), RNA was extracted from the cells using a hot phenol protocol and Phase Lock Gel Tubes (5 Prime), quantified using a Biophotometer and analyzed by agarose gel electrophoresis to check for integrity. In concluding this experiment, the total purified RNA samples will be shipped to Germany for dRNA-seq. Once there, programmers will map out the RNA sequence, and upload it to the Gene Expression Database (GenExpDB). GenExpDB will be used to investigate the differential gene expression in each RNA sample. We will be comparing the

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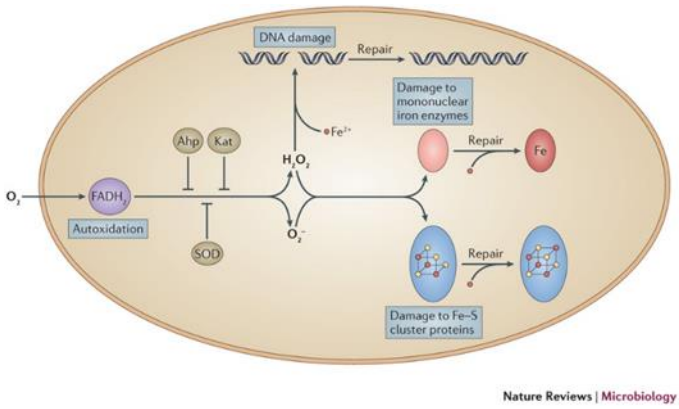

Figure 1 *- An overview of ROS-induced damage caused in E. coli*

transcriptomes of the oxidative stress sample(s) with an rpoS mutant and untreated controls.

Results

The research hypothesis of this experiment was that the exposure of E. coli cultures to excess H_2O_2 will induce the expression of H_2O_2-specific genes involved in ROS neutralization, and a large-scaled and prompt re-programming of gene expression occurs when a steady-state growth is disturbed (Chang *et al.* 2002). Also, the differential RNA Sequencing (dRNA-seq) analysis of RNA extracted from an *E. coli* rpoS mutant (ΔrpoS) exposed to H_2O_2 will identify gene promoters specifically activated that are independent of the rpoS-mediated general stress response. However, after conducting this portion of the experiment as seen in Figure 2, we expect that treatment with 50 µg/mL H_2O_2 did not induce a significant change in the growth rate of *E. coli* grown in the presence of 200 $\mu g \cdot mL^{-1}$ H_2O_2 experienced a growth arrest at ≅ 910 minutes (Figure 2). This is consistent with cell cycle arrest, which permits damaged DNA to be repaired while the cells are not dividing. Also, inducing oxidative stress with H_2O_2 will significantly upregulate OxyR-dependent transcripts in WT and rpoS mutant (ΔrpoS) *E. coli* strains. Knockouts in OxyR-induced genes will impair the ability of *E. coli* to neutralize H_2O_2.

Discussion

The growth curves of *E. coli* BW39452 (ΔrpoS) will be performed in the presence of 50 µg/mL H_2O_2, and total RNA will be extracted. By using GenExpDB, an analysis of the total RNA extracted from BW38028 and BW39452 strains treated with 50 µg/mL H_2O_2 should reveal significant quantitative evidence supporting ROS-induced cell cycle arrest and identify H_2O_2-elicited pathways which are differentially expressed during the *E. coli* oxidative stress response. The methods used to conduct this research experiment have been conducted in a similar fashion within previous research done by other scientists, such as Chang D.E. The results of this experiment will contribute to the scientific knowledge of the genetic classification within *E. coli* genes, and their response to transient growth inhibition following H_2O_2-induced oxidative stress. This experiment will also provide a further analysis into the bacterial cells comprehensive system response to transient and stationary phase growth arrest (Chang et al. 2002). In our experiment, gene knockouts will be made within the *E. coli* to disrupt the H_2O_2-specific pathways. These mutants will be exposed to 50 µg/mL H_2O_2 to test their regulatory role in the oxidative stress response.

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